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(54) Title: COMPOSITION AND METHOD FOR MODULATING DENDRITIC CELL–T CELL INTERACTION

(57) Abstract

The present invention relates to the use of a compound that binds to a C–type lectin on the surface of a dendritic cell, in the preparation of a composition for modulating, in particular reducing, the immune response in an animal, in particular a human or another mammal. The composition in particular modulates the interactions between a dendritic cell and a T–cell, more specifically between a C–type lectin on the surface of a dendritic cell and an ICAM receptor on the surface of a T–cell. The compositions can be used for preventing/inhibiting immune responses to specific antigens, for inducing tolerance, for immunotherapy, for immunosuppression, for the treatment of auto–immune diseases, the treatment of allergy, and/or for inhibiting HIV infection. The compound that binds to a C–type lectin is preferably chosen from mannose, fucose, plant lectins, antibiotics, sugars, proteins or antibodies against C–type lectins. The invention also relates to such antibodies, and to a method for isolating dendritic cells using such antibodies.

Titel: Composition and method for modulating dendritic cell-T cell interaction.

The present invention relates to compositions and a method for modulating, in particular increasing or reducing, the immune response in an animal, such as a human or another mammal.

In one embodiment, the invention relates to compositions and a method for modulating, and in particular reducing, the adhesion of dendritic cells to T cells.

More specifically, this embodiment of the invention relates to compositions and a method for modulating, and in particular reducing, the adhesion of C-type lectin receptors on the surface of dendritic cells to the ICAM-receptors on the surface of T cells. By modulating this adhesion, both dendritic cell-T cell interactions, such as cluster formation and antigen presentation, as well as for instance primary T cell responses dependant thereon, can be influenced, resulting in a modulation of the immune response.

The compositions and method of the invention can therefore be used to alter immune responses to specific antigens as well as immune responses caused by disorders of the immune system, such as may occur in auto-immune diseases or in allergy.

In a further embodiment, the method of the invention can further be used in the treatment of HIV-infections and similar disorders of the immune system, as well as to modulate the immune response to grafts or after transplant.

In another embodiment, the invention relates to compounds, compositions and methods for modulating, and in particular increasing, the immune response in an animal, such as a human or another mammal.

More specifically, in this embodiment, an immune response against a specific antigen is generated, increased or promoted by presenting said antigen or an antigenic part thereof to dendritic cells in a form that can bind to the C-type lectin receptors on the surface of dendritic cells. The antigen presented in this manner enters the dendritic cell, which in turn presents the antigen to the T-cells, thereby causing an immune response, or an increased immune response, against

Curtis et al., in *Proc.Natl.Acad.Sci.* USA, 89 (1992), p. 8356-8360, as well as in WO 93/01820, describe a non-CD4 gp120 receptor isolated and cloned from human placenta tissue. This gp120 receptor is expressed on mammalian cells which do not exhibit high levels of CD4, such as placenta, skeleton muscle, brain, neural and mucosal cells, as well as other tissues and cells including colon, thymus, heart, T cells, B cells and macrophages (but not in the liver or the kidney). The amino acid sequence of the C-type lectin gp120 receptor disclosed in SEQ ID's no. 1 and 2 of WO 93/01820 has a high degree of sequence homology (>98%) with the C-type lectins that are now found to be present on dendritic cells.

Curtis and WO 93/01820 further discuss the role this C-type lectin receptor plays in the infection of the aforementioned cells/tissues with HIV, i.e. by binding the major HIV envelope glycoprotein gp120 prior to internalization of the virion into the cell. It was found that inhibition of the C-type lectin gp120 receptor can reduce or inhibit HIV infection of these cells/tissues. As suitable inhibitors, WO 93/01820 discloses mannose carbohydrates, fucose carbohydrates, plant lectins such as concanavalin A, specific antibiotics such as pradimicin A, and sugars such as N-acetyl-D-glucosamine and galactose (which however are described as less potent). These compounds and compositions containing them are used either *in vitro* or *in vivo* to inhibit the binding of HIV to the cell surface.

WO 93/01820 further discloses that binding of HIV to COS-7 cells can be inhibited by pre-incubation of gp120 with an anti-gp120 monoclonal antibody (named "antibody 110.1"). However, this antibody is not directed against the C-type lectins, but against the gp120 protein.

However, neither Curtis nor WO 93/01820 mentions or suggests the presence of such a C-type lectin on dendritic cells, nor do these references mention or suggest their role in dendritic cell - T cell interaction during the initial stages of an immune response.

WO 95/32734 describes FcγRII (CD32) bridging (or crosslinking) compositions and their use in modulating the immune response to specific antigens. This reference is based upon the finding that the bridging of FcγRII (CD32) molecules on antigen presenting cells (APCs) impaires the expression of

HIV. It is now generally believed that HIV converts the normal trafficking process of DC to gain entry into lymph nodes and access to $CD4^+$ T cells, as was demonstrated *in vivo* using primary simian immunodeficiency virus infection of macaque as a model system (Spira et al., 1996)(Joag et al., 1997). Productive infection of DC with HIV-1 has been reported by several investigators (Granelli-Piperno et al., J Virol 72(4), 2733-7, 1998) (Blauvelt et al., Nat Med 3(12), 1369-75. 1997.) and substantial evidence indicates that DC pulsed with HIV-1 in vitro can induce a vigorous infection when co-cultured with T cells (Cameron et al., Science 257(5068), 383-7, 1992). Although it is still unclear whether a similar scenario occurs in HIV infected individuals, HIV-1 transmission from DC to T cells could contribute to the $CD4^+$ T cell depletion observed in AIDS. Studying HIV-DC interactions should contribute to the understanding of early events of HIV infection and will hopefully lead to strategies aimed at blocking early events in transmission. For a further discussion, reference is also made to WO 95/32734 and WO 96/23882.

DC are unique in their ability to interact with and activate resting T cells. However, prior to the present invention, it was largely unknown how DC-T cell contact is initiated and regulated. Herein, the role of ICAM-3 in DC-T cell interactions is investigated. It is demonstrated that although DC strongly adhere to ICAM-3, this adhesion is not mediated by LFA-1, αD or any other integrin. In the search for this novel ICAM-3 receptor on DC a C-type lectin receptor was cloned, designated DC-SIGN, that is preferentially expressed by DC. Besides its prominent role in DC-T cell clustering and initiation of T cell responses we discovered that DC-SIGN is a major HIV-1 receptor involved in infection of DC and subsequent HIV-1 transmission to T cells. Thus HIV-1 and resting T cells exploit a similar highly expressed receptor to interact with DC.

In a first aspect, the invention relates to the use of a compound that binds or can bind to a C-type lectin on the surface of a dendritic cell, in the preparation of a composition for modulating, in particular reducing, the immune response in a animal, in particular a human or another mammal.

In particular, this aspect of the invention relates to the use of a compound

all interactions that rely on contact between dendritic cells and T-cells, by which is meant either direct cell-to-cell contact or close proximity of dendritic cells and T cells.

Such further interactions include, but are not limited to, processes involved in generating an immune response, in particular during the initial stages of such a response, such as primary sensitation/activation of T-lymphocytes, (i.e. presentation of antigen and/or MHC-bound peptides to T-cells) and co-stimulation of T cells; as well as processes such as chemical signalling, endocytosis and trans-epithelial transport. For a discussion of dendritic cell-T cell interactions in general, all of which may be influenced by the compositions of the invention, reference is made to the discussion below as well as to WO 95/32734 and WO 96/23882.

The compositions of the invention can therefore be used to influence the immunomodulatory ability of dendritic cells; to modulate, and in particular reduce, dendritic cell-mediated (primary) T cell responses, and/or generally to influence, and in particular inhibit, the immune system.

Some specific applications include preventing or inhibiting immune responses to specific antigens; inducing tolerance; immunotherapy; immunosuppression, for instance to prevent transplant rejection; the treatment of auto-immune diseases such as thyroiditis, rheumatoid arthritis, systemic lupus erythematosus (SLE), multiple sclerosis and auto-immune diabetes; and the prevention or treatment of allergies.

The compositions of the invention may also modulate the activation of other receptors on T cells which are dependant upon the adhesion or close proximity of dendritic cells to T cells. Furthermore, the finding of the invention that a C-type lectin on dendritic cells binds to the ICAM receptors on T cells may open up new strategies or possibilities for influencing the interaction between dendritic cells and T cells, and thereby for modulating the immune system in general.

Furthermore, the compositions of the invention can be used to prevent or reduce the transfer of matter from dendritic cells to T cells, such as chemicals, signalling factors such as chemokines and/or interleukines, etc., and in particular

mannose; fucose carbohydrates such as L-fucose; plant lectins such as concanavalin A; antibiotics such as pradimicin A; sugars such as N-acetyl-D-glucosamine and galactose (which however are described as less potent); as well as suitable peptidomimetic compounds and small drug molecules, which can for instance be identified using phage display techniques. Furthermore, proteins such as gp120 and analogs or fragments thereof or similar proteins with binding capacity to C-type lectins on dendritic cells may be used, as well as isolated ICAM-receptors and analogs thereof, including part or fragments thereof. Such parts or fragments should then preferably still be such that they can bind to the C-type lectins on the surface of dendritic cells.

However, the use of carbohydrates is usually less desired from a therapeutic point of view, as such they can be rapidly metabolized *in vivo*. Also, the use of plant lectins such as concanavalin A and pradimicin antibiotics can have disadvantages in a therapeutic setting, in particular when treating patients with auto-immune disorders and/or HIV-infections.

Preferably, one or more physiological tolerable and/or pharmaceutically acceptable compounds are used, such as defined in WO 93/01820. For instance, the use of gp120 or derivatives thereof may cause undesired side effects, in particular on the nervous system (vide WO 93/01820).

Therefore, according to the invention, preferably an antibody directed against a C-type lectins as present/expressed on the surface of a dendritic cell, or a part, fragment or epitope thereof, is used. As used herein, the term antibodies includes *inter alia* polyclonal, monoclonal, chimeric and single chain antibodies, as well as fragments (Fab, Fv, Fa) and an Fab expression library. Furthermore, "humanized" antibodies may be used, for instance as described WO 98/49306.

Such antibodies against the C-type lectins of the invention can be obtained as described hereinbelow or in any other manner known per se, such as those described in WO 95/32734, WO 96/23882, WO 98/02456, WO 98/41633 and/or WO 98/49306.

For instance, polyclonal antibodies can be obtained by immunizing a suitable host such as a goat, rabbit, sheep, rat, pig or mouse with a C-type lectin

European Collection of Cell Cultures under (provisional) ECACC accesion numbers 990400818 and 99040819, respectively.

The invention also relates to a method for producing an antibody, preferably a monoclonal antibody, against the C-type lectins on dendritic cells, more specifically against the peptide with the amino acid sequence shown in (or encoded for) by SEQ ID no's 1 and 2 and Figure 9 or (an antigenic) part thereof, said method comprising cultivating a cell or a cell line that produces said antibody and harvesting/isolating the antibody from the cell culture.

Neither (monoclonal) antibodies against the C-type lectins on dendritic cells, nor cells or cell lines that produce such antibodies, have to date been described in the art, and it is envisaged that the novel antibodies of the invention will have broad applicability (i.e. besides the pharmaceutical/therapeutic uses disclosed herein). Some of these application -which form yet another aspect of the invention- will be clear to the skilled person from the disclosure herein.

For instance, the antibodies of the invention can be used to detect the presence of (and thereby determine the expression of) C-type lectins in or on tissues or whole cells, as well as the detect the presence of C-type lectins in other biological samples such as cell fragments or in cell preparations. The information thus obtained can then (also) be used to determine whether the method or compostions of the invention can be applied to such tissues or cells. The antibodies of the invention could also be used to detect (qualitatively and/or quantitatively), isolate, purify and/or produce dendritic cells, for instance in/from biological samples, including biological fluids such as blood, plasma or lymph fluid; tissue samples or cell samples such as bone marrow, skin tissue, tumor tissues, etc; or cell cultures or cultivating media.

For instance, the few methods presently available for isolating/producing dendritic cells from biological samples - such as the method described in US-A-5,643,786, comprising leukapherese followed by fluorescence-activated cell-sorting - are very cumbersome multi-step procedures that provide only low yields and heterogenous samples. As a result, the limited availability of dendritic cells has severely hindered research into this important class of cells.

formation and development) and auto-immune diseases (including for instance rheumatoid arthitis).

For a further description of the methods and techniques known per se in which the antibodies of the invention can be used, reference is made to the general textbooks, such as D.P. Sites, A.I. Terr, T.G. Parslow: "Basic and clinical immunology", 8th Ed., Prentice-Hall (1994); I. Roitt, J. Brostof, D. Male: "Immunology", 2nd. Ed., Churchill Livingstone (1994); all incorporated herein by reference. Particular reference is made to the general uses of antibodies and techniques involved therein as mentioned in sections 2.7 to 2.17 of the general reference work by Janeway-Travers: "Immunobiology, the immune system in health and disease", Third Edition.

A composition of the invention may contain two or more of the above-mentioned active compounds, or such compounds may be used in combination. For instance, an anti-C-type lectin antibody can be formulated with mannose or fucose carbohydrates, lectins and/or antibiotics such as pridamicin A, whereby a synergistic effect may be obtained.

The compositions of the invention may also contain or be used in combination with known co-inhibitory compounds, such as anti-LF3A; as well as other active principles known per se, depending upon the condition to be treated. For instance, the compositions of the invention may be formulated or used in combination with immunosuppressants (i.e. for preventing transplant rejection), immunomodulants, antibiotics, auto-antigens or allergens (for instance as described in WO 95/3234 or WO 96/23882), Tumor Necrosis Factor (TNF), and anti-viral agents such as anti-HIV agents and CD4 inhibitors including CD4 directed antibodies such as Leu-3A, whereby again a synergistic effect can be obtained.

The compositions of the invention can further be formulated using known carriers and/or adjuvantia to provide a pharmaceutical form known per se, such as a tablet, capsule, powder, freeze dried preparation, solution for injection, etc., preferably in a unit dosage form. Such pharmaceutical forms, their use and administration (single or multi dosage form), as well as carriers, excipients, adjuvantia and/or formulants for use therein, are generally known in the art and

amount that the transfer of HIV from infected dendritic cells to non-infected T cells is inhibited.

In a further aspect, the invention is used to modulate, and in particular generate, increase and/or promote, an immune response in an animal, such as a human or another mammal, against a specific antigen or combination of antigens, by presenting said antigen(s) or one or more antigenic parts thereof to dendritic cells in a form that can bind to the C-type lectin receptors on the surface of dendritic cells. The antigen(s) presented in this manner enter(s) the dendritic cell, which then in turn presents the antigen to the T-cells, thereby causing an immune response against the antigen(s).

With "a form that can bind to the C-type lectin receptors on the surface of dendritic cells" is generally meant that the antigen or antigenic fragment is attached to a compound, ligand or residu that can bind to a C-type lectin on the surface of a dendritic cell, such as the compounds/ligands mentioned above or a part thereof. Said attachment can for instance be by (preferably covalent) binding, ligand-ligand interaction, complexing, ligation, fusion of proteins (e.g. through expression of said fusions), or by any other type of physical or chemical interaction or bond that enables the antigen to be presented to a dendritic cell in conjunction with the ligand for the C-type lectin, i.e. combined into a stable or semi-stable entity.

For instance, the antigen can be provided with the abovementioned mannose and fucose carbohydrates as covalently bound groups or side-chains; can be covalently attached to plant lectins such as concanavalin A or antibiotics such as pradimicin A; or can be provided with sugar residues such as N-acetyl-D-glucosamine and galactose (which however is less preferred), all of which serve to "direct" the antigen to the dendritic cell.

Preferably, the antigen is attached to (e.g. fused with or covalently bonded to) to a protein that can bind to or serve as a ligand for the C-type lectins, such as gp120 and analogs thereof or the ICAM-receptors and analogs thereof, or to a part of fragment of such a protein. Alternatively, the antigen can be attached to (e.g. fused with or covalently bonded to) an antibody directed against the C-

of a composition for modulating, in particular generating, increasing and/or promoting, an immune response in a animal, in particular a human or another mammal, against said antigen.

These combinations (e.g. in the form of a complex, a chemical substance or entity, or a fused protein or protein structure), which as such form another aspect of the invention, can again be formulated and administered in a manner known per se, such as described above.

In all the above methods en embodiments, the compounds/compositions used will be administered in a therapeutically effective amount, for which term reference is generally made to WO 93/01820, WO 95/32734 and/or WO 96/23882. The administration can be a single dose, but is preferably part of a multidose administration regimen carried out over one or more days, weeks or months.

All terms used herein have the normal meaning in the art, for which reference can be made to *inter alia* the definitions given in WO 93/01820, WO 95/32734, WO 96/23882, WO 98/02456, WO98/41633 and/or WO 98/49306, analogously applied.

Furthermore, although the invention is described herein with respect to the specific 44kDa C-type lectin receptor disclosed herein, it is not excluded that other, generally similar C-type lectins, including natural variants of the sequence of SEQ ID no.1 and Figure 9, may also be present on dendritic cells and/or may be involved in dendritic cell - T cell interaction. Such variants will usually have a high degree of amino acid homology (more than 80% to more than 90%) with, and/or be functionally equivalent to the specific C-type lectin disclosed herein. Also, any such receptor will generally display properties similar to those as described herein; in particular that inhibition of this receptor, either by carbohydrate inhibitors or specific antibodies, will lead to an alteration of dendritic cell/T-cell interaction. Any such variant receptors should however be distinguished from the C-type lectin receptor disclosed in WO 96/23882, inhibition of which does not result in inhibition of the interaction of dendritic cells and T-cells.

The invention will now be further illustrated by means of the Experimental Part given hereinbelow, as well as the Figures, in which:

Dendritic cells (DC) capture antigens and migrate to secondary lymphoid tissues where they present antigens to naive T cells. HIV-1 subverts this unique capacity to gain access to $CD4^+$ T cells. In the invention, a DC specific C-type lectin was cloned, designated DC-SIGN, that not only binds to ICAM-2 and/or ICAM-3 with high affinity but is also able to bind HIV-1. Also, anti-DC-SIGN antibodies were developed that not only inhibit transient DC-T cell interactions and DC induced T cell proliferation but also effectively inhibit HIV-1 infection of DC. These findings not only have important consequences for the understanding on CD4-independent HIV entry into DC but also shed new light on the role of DC-SIGN in initiating primary immune responses.

Example 1: Adhesion of DC to ICAM-3 is not mediated by integrins

The role of ICAM-3 mediated adhesion in first DC-T cell contact was investigated. Exploiting a novel flowcytrometric adhesion assay involving ICAM-3-Fc chimera coated fluorescent beads (Geijtenbeek et al.), the capacity of DC, resting peripheral blood lymphocytes (PBL) and monocytes to bind to this integrin ligand was tested. Immature DC, obtained after culturing of monocytes for 7 days in the presence of IL-4 and GM-CSF, strongly bind ICAM-3 without prior activation of β2 integrins (72%, Figure 1A). Figure 1 demonstrates that the adhesion of DC to ICAM-3 is Ca^{2+}-dependent and integrin-independent: in Figs. 1A, B and C one representative experiment of at least 3 is shown (SD<5%).

- 1A: Spontaneous adhesion of leukocytes to ICAM-1 and ICAM-3. Freshly isolated PBL, monocytes and DC were incubated for 30 min. at 37°C with either ICAM-1Fc or ICAM-3Fc fluorescent beads. After washing, the percentage of cells that bound beads was determined by flowcytometry.
- 1B: Adhesion of leukocytes to ICAM-3 after activation of β2-integrins. Binding of fluorescent ICAM-3Fc beads was measured after 30 min. at 37°C in the presence of either PMA 980 nM) or the activating anti-β2-integrin antibody KIM185 (10μg/ml). Inhibition of the LFA-1 specific adhesion after PMA activation was determined in the presence of the blocking anti-LFA-1 antibody NKI-L15 (20μg/ml).

D1 and AZN-D2 inhibit adhesion of DC to ICAM-3 and recognize an antigen that is specifically expressed by DC:

- 2A: The monoclonal antibodies AZN-D1 and AZN-D2 (20 μg/ml) block adhesion of DC but not that of freshly isolated monocytes to fluorescent ICAM-3Fc beads. A representative experiment of at least 3 experiments is shown (SD<5%).
- 2B: DC-SIGN expression increased during DC development. DC were cultured from monocytes in the presence of GM-CSF and IL-4. At different timepoints the developing DC were analyzed for expression of the monocyte marker CD14, β2 integrin LFA-1 and DC-SIGN. Cells were gated on forward-side scatter and the mean fluorescence is shown in the top right corner of the histograms. A representative experiment out of 3 is shown.
- 2C: DC developing from monocytes, in the presence of GM-CSF and IL-4, increasingly bind to ICAM-3 in a DC-SIGN dependent manner. At different time points during culturing cells were harvested and incubated with fluorescent ICAM-3Fc beads in the presence of the blocking anti-β2-integrin antibody AZN-L19 or the AZN-D1 antibody (20μg/ml). Adhesion was determined as described in Fig. 1A. AZN-D2 inhibited adhesion to ICAM-3 similar to AZN-D1 (results not shown). A representative experiment out of 3 is shown (SD<5%).
- 2D: Relative contribution of β2-integrins and DC-SIGN mediated adhesion to ICAM-3 by developing DC. Relative contribution is calculated from the inhibition of adhesion in the presence of AZN-D1 or AZN-L19 as described in Fig. 2C.

Using AZN-D1 antibodies in flowcytometric analyses it was demonstrated that DC-SIGN is not expressed by monocytes (Figure 2B). Cells expressing DC-SIGN can already be detected after 1 day of culture. The expression level of DC-SIGN increases during culture (Figure 2B). The expression of the monocyte marker CD14 gradually decreases during culture and at day 7 only a low CD14 expression is observed (Figure 2B). Further flowcytometric analyses demonstrated that at day

followed by autoradiography. The migration of the molecular weight markers is indicated on the left. The arrows indicate the α-chains of LFA-1 (αL, 180 kDa), MAC-1 (αM, 165 kDa) and p150,95 (αX, 150 kDa), the β2 integrin chain (95 kDa) and DC-SIGN (44 kDa). Similar results were obtained in 3 other experiments.

- 3B: Schematic presentation of DC-SIGN isolated from human DC. The two boxed peptides (aminoacid positions 296-306 and 187-197 of the human placenta gp120 binding C-type lectin () were identified by internal peptide sequencing of immunoprecipitated DC-SIGn using Edman degradation. The cDNA encoding DC-SIGN was isolated from DC. The deduced amino acid sequence is 100% identical to that of the human placenta gp120 binding C-type lectin (). The transmembrane region, the lectin domain and the seven complete and eight partial repeats (R1-R8) are indicated.

Analysis of the immunoprecipitate on a non-reducing SDS-PAGE gel shows that DC-SIGN exists as a monomer (results not shown). Furthermore, using ICAM-3-Fc coated beads also a 44 kDa protein could be extracted from the DC lysate whereas in the presence of blocking anti-DC-SIGN antibodies this protein could not be precipitated with ICAM-3-Fc coated beads (results not shown). These findings demonstrate that DC-SIGN is expressed by DC as a 44 kDa protein under reducing conditions. The observation that ICAM-3 Fc coupled beads only extracted a 44 kDa protein out of the DC lysate indicates that DC-SIGN has a high affinity for ICAM-3, much higher than LFA-1 or αDβ2 which are also expressed by DC (Figure 3A) and have been reported to bind ICAM-3 (Vandervieren et al., Immunity. *3*, 683-690, 1995). Since very low amounts of LFA-1 are immunoprecipitated in comparison to DC-SIGN (Figure 3A, lane 1 and 3) this confirms that DC-SIGN is more abundantly expressed by DC than LFA-1. Together, these data demonstrates that DC-SIGN is a single polypeptide of 44 kDa and is the primary receptor for ICAM-3 on DC.

Example 4: DC-SIGN is identical to the human HIV gp120 binding C-type

About 30% of the transfected COS7 cells are stained with anti-DC-SIGN-antibody and therefore express DC-SIGN. Moreover, the COS7-DC-SIGN cells are able to bind ICAM-3 whereas mock transfected COS7 cells are unable to bind ICAM-3 (Figure 4B). Binding of DC-SIGN expressed by COS7 could be completely inhibited by antibodies against ICAM-3 and DC-SIGN, and was Ca^{2+} dependent since EGTA blocks adhesion completely (Figure 4B).

It as concluded that the ICAM-3 binding receptor expressed by DC (DC-SIGN) is identical to the placenta HIV gp120 binding C-type lectin (Curtis et al., 1992), a type II transmembrane protein consisting of 404 aa with three distinct domains. The N-terminal cytoplasmic domain of 40 aa residues is separated by a hydrophobic stretch of 15 aa from a region which consists of seven complete and one incomplete tandem repeat of nearly identical sequence. The remaining C-terminal region (Cys253-Ala404) shows homology to Ca^{2+}-dependent (C-type) lectins (Figure 3B).

Example 5: DC-SIGN is specifically expressed by DC.

Flowcytometric analysis of an extensive panel of hematopoietic cells with the AZN-D1/D2 antibodies demonstrates that the antigen is preferentially expressed by DC (Table 1). All the hematopoietic cells tested were negative for DC-SIGN expression except for DC. Furthermore, a RT-PCR analysis confirms that the mRNA encoding DC-SIGN is specifically transcribed in DC which is in accordance with the expression pattern of the DC-SIGN protein (Table 1).

To further investigate the expression of DC-SIGN in-vivo, immunohistochemical analysis of secondary lymphoid tissues with the anti-DC-SIGN antibodies was performed. These tissues are known to contain dendritic cells. Sections of tonsils and lymph nodes contained DC-SIGN expressing cells, which were predominantly observed in the T cell area of both tonsils and lymph nodes (Figure 5). Figure 5 shows the tissue distribution of DC-SIGN: Immunohistochemical analysis of the expression of DC-SIGN in tonsils and lymph node sections (OMx100). Sections were fixed with acetone and the nuclear staining was performed with Hematein. Staining of DC-SIGN was performed with

and the heterotypic cell clustering was measured flow-cytometrically. A representative experiment of 2 experiments is given.

- 6C: Dynamic cell clustering of DC with resting PbL is mediated by DC-SIGN. DC ($50x10^3$ cells) were pre-incubated with/without the anti-DC-SIGN antibodies AZN-D1 and AZN-D2 (10 μg/ml) for 10 min. at RT. Allogeneic PBL ($1x10^6$ cells), labeled with the fluorescent dye Calcein-A ($25\mu g/10^7$ cells/ml for 30 min. at 37°C), were added and the cell mixture was incubated at 37°C. The clustering was measured by flow-cytometry. A representative experiment out of 2 is shown.

- 6D: The DC-SIGN-ICAM-3 interaction is important in DC-induced T-cell proliferation. Allogeneic responder T-lymphocytes ($100x10^3$) were added to DC-stimulators ($1.5x10^3$) in the presence of blocking antibodies (20 μg/ml) against LFA-3 (TS2/9) and DC-SIGN (AZN-D1, AZN-D2). The cells were cultured for 4 days. On day 4 the cells were pulsed for 16 h with [^{3}H]methyl-thymidine and the uptake was determined. The results are expressed as the mean percent of CPM from triplicate wells.

DC-SIGN dependent clustering is transient, with a maximum at 60 minutes indicating that DC-SIGN-ICAM-3 interactions may be actively regulated by the DC. Furthermore, this phenomenon allows DC to transiently interact with multiple naive T cells until the interaction is strengthened after TCR engagement.

To test this it was investigated whether clustering of DC to T cells is mediated by DC-SIGN and whether this interaction is also transient. DC were incubated with resting allogeneic T cells (DC:T cell, 1:20) and the DC-T cell clustering was determined. As shown in Figure 6B, the clustering of DC with T cells is transient and reaches a maximum after 20 min (32%). Furthermore, the DC-T cell interaction can be inhibited (approximately 50%) by anti-DC-SIGN antibodies suggesting that the DC-T cell clustering is also mediated by other surface receptors. Thus, the DC-T cell clustering is indeed transient and partly mediated by DC-SIGN/ICAM-3 interactions. Similarly, Figure 8 shows that DC-SIGN binds not only with K562 cells expressing cDNA encoding ICAM-3, but also to K562 cells expressing cDNA encoding ICAM-3, and that said binding can

HIV-1 replication is inhibited for more than 75%, as shown at day 3 and 5 of DC-T cell co-culture (Figure 7). When DC were incubated with anti-DC-SIGN antibodies after pulsing with HIV-1, efficient HIV-1 replication was still observed in the DC-T cell co-culture (Figure 7). These findings demonstrate that anti-DC-SIGN antibodies block HIV-1 infection through inhibition of HIV-1 binding to DC and not the HIV-1 transmission from DC to T cells, indicating that DC-SIGN act as a major receptor for HIV-1 on DC. Thus, DC-SIGN is highly expressed on DC and functions as a DC specific receptor for both ICAM-3 and HIV-1.

From the above experimental results, it can *inter alia* be concluded that the initial interaction of DC with T lymphocytes is antigen-independent and transient. This transient nature provides DC with the capacity to interact with a multitude of T cells until a productive TCR engagement is made. Until now, the mechanism by which this transient process is initiated has been unclear. Herein, it is demonstrated that the interaction of a novel DC specific receptor, DC-SIGN, with ICAM-3 mediates this transient DC-T cell interaction. DC-SIGN is abundantly expressed by DC and it was shown that DC-SIGN serves as a major HIV-1 receptor on DC.

An important role for DC during the course of HIV-1 infection is the ability to spread HIV-1 to T cells, promoting extensive replication that leads to the death of CD4+ T cells (Cameron et al., 1992; Cameron: AIDS Res Hum Retroviruses 10(1), 61-71, 1994). Productive HIV-1 infection of DC has been clearly demonstrated and depends on the development stage of the DC (Granelli-Piperno et al., 1998). Immature DC, cultured from monocytes in the presence of IL-4 and GM-CSF, are productively infected by M-tropic HIV-1 strains (Granelli-Piperno et al., 1996; Granelli-Piperno et al., 1998)(Blauvelt et al., 1997) whereas both M- and T-tropic HIV-1 entry into mature DC does not lead to a productive infection (Granelli 1998). However, HIV-1 entry into both types of DC does lead to an explosive replication upon co-culturing with either resting or activated T cells (Granelli 1998, 1999). The initial events in HIV-1 infection of target cells include receptor binding and membrane fusion. This process is initiated by the high affinity binding of the envelope glycoprotein gp120 to CD4. However, CD4

effort has gone into the generation of antibodies that are directed against DC lineage specific cell surface molecules. So far only a few antibodies have been generated which recognize human DC specific antigens ((Hock et al., Immunol. *83*, 573-581, 1994), (de Saint-Vis et al., Immunity 9(3), 325-36, 1998)(Hart et al., 1997). DC-SIGN can now been added to this list of human DC specific antigens since it was demonstrated herein that at the protein as well as mRNA level, of all hematopoietic cells tested, only DC express DC-SIGN (Table 1). In situ DC-SIGN is exclusively expressed by DC subsets present in the T cell area of tonsils and lymph nodes. These mature DC are very potent in the activation of naive T cells. Therefore, DC-SIGN expression in situ correlates with its function as an important mediator of DC-T cell clustering and subsequent T cell activation.

Activation of resting T lymphocytes by antigen presenting cells is a critically important step in the acquired immune response. Located in most tissues, DC capture and process antigens, and migrate to lymphoid tissues where they interact with and activate naive antigen-specific T cells. T cells are directed by chemokines to these sites of antigen presentation. Recently, a DC specific chemokine DC-CK1 was identified which specifically attracts naive T cells to immune initiation sites (Adema et al., Nature *387*, 713-717, 1997). Upon arrival in secondary lymphoid tissues, T cells interact with DC and activation occurs after TCR recognition of peptides bound to MHC molecules. However, since the affinity of the TCR for the antigen presented by MHC molecules is very low and the number of specific MHC-peptide complexes on APC is limited, the interaction of TCR with antigen is usually insufficient to drive the formation of intimate membrane contact between DC and T-lymphocyte necessary for full activation.

To date LFA-1 was the most important receptor for ICAM-3 on DC. However, its role in ICAM-3 binding has now become disputable due to the discovery herein of DC-SIGN. It was demonstrated that adhesion of DC to ICAM-3 is completely mediated by DC-SIGN. DC-SIGN is more abundantly expressed by DC than LFA-1 (Figure 2B). Furthermore, LFA-1 is inactive on DC (Figure 2C) and its affinity for ICAM-3 is much lower than that of DC-SIGN for ICAM-3. These data clearly demonstrate that DC-SIGN is the primary receptor for

Ex. 9A: Antibodies

The following antibodies were used: KIM185 (anti-β2 integrin, (Andrew et al., Eur.J.Immunol. *23*, 2217-2222, 1993), AZN-L19 (anti-β2 integrin,), NKI-L15 (anti-αL, (Keizer et al., Eur.J.Immunol. *15*, 1142-1147, 1985)), AIIB2 (anti-β1 integrin, (Da Silva et al., J.Immunol. *143*, 617-622, 1989)), CBR-IC3/1 and CBR-IC3/2 (anti-ICAM-3 (de Fougerolles et al., J.Exp.Med. *177*, 1187-1192, 1993)), CD14 (WT14 ()), CD4 (wt4 ()). The anti-DC-SIGN antibodies AZN-D1 and AZN-D2 were obtained by immunizing BALB/c mice with DC and subsequently screening the hybridoma supernatants for the ability to block adhesion of DC to ICAM-3 as measured by the fluorescent beads adhesion assay.

Ex. 9B: Cells

DC were cultured from monocytes as described (Sallusto and Lanzavecchia, J.Exp.Med. *179*, 1109-1118, 1994; Romani et al., J.Exp.Med. *180*, 83-93, 1994). Briefly, monocytes were isolated from fresh PBMC by an adherence step. The monocytes were cultured in the presence of IL-4 (Schering-Plough, Brussels, Belgium; 500 U/ml) and GM-CSF (Schering-Plough, Brussels, Belgium; 1000 U/ml) for 7 days. At day 4 fresh cytokines were added. At day 7 the phenotype of the cultured DC was confirmed by flowcytometric analysis of the expression of MHC class I and II, CD1a, p150,95 and CD80. Stable K562 transfectants expressing ICAM-3 (K562-ICAM-3) were generated by transfection of K562 with 10 μg PCRII ICAM-3 R1 plasmid (gift from Dr D. Simmons) and 2 μg PGK-hyg vector (te Riele et al 1990) by electroporation as described (Lub et al., Mol.Biol Cell *8*, 719-728, 1997). Resting T cells (>90% CD3 positive) were obtained by centrifugal elutriation of PBMC from bone marrow of healthy donors, as described (Figdor et al., J.Immunol.Methods *68*, 73-87, 1984).

Ex. 9C: Radiolabeling, immunoprecipitation and protein sequence analysis.

Cells were surface labeled with $Na^{125}I$ (Amersham, Buckinghamshire, UK) through the lactoperoxidase method (Pink and Ziegler, 1979, in: *Research Methods in Immunology*, L. Lefkovits and B. Pernis, eds. (New York: Academic Pres), pp.

previously (Geijtenbeek et al., 1999 submitted). Briefly, 20 µl streptavidin (5 mg/ml in 50 mM MES-buffer) was added to 50 µl TransFluorSpheres. 30 µl EDAC (1.33 mg/ml) was added and the mixture was incubated at RT for 2h. The reaction was stopped by the addition of glycin to a final concentration of 100 mM. The streptavidin-coated beads were washed three times with PBS (50 mM phosphate, 0.9% NaCl pH 7.4) and resuspended in 150 µl PBS, 0.5% BSA (w/v). The streptavidin-coated beads (15 µl) were incubated with biotinylated goat-anti-human anti-Fc Fab2 fragments (6 µg/ml) in 0.5 ml PBA for 2 hours at 37°C. The beads were washed once with PBS, 0.5% BSA and incubated with human IgG1 Fc fused ligands (ICAM-1 Fc, VCAM-1 Fc; 250 ng/ml) in 0.5 ml overnight at 4°C. The ligand-coated beads were washed, resuspended in 100 µl PBS, 0.5% BSA and stored at 4°C. ICAM-1 Fc and ICAM-3 Fc consist of the extracellular part of the protein fused to a human IgG1 Fc fragment (provided by Dr D. Simmons). The fluorescent beads adhesion assay was performed as described by Geijtenbeek et al. (submitted). Briefly, cells were resuspended in Tris-Sodium-BSA buffer (20 mM Tris-HCl pH 8.0, 150 mM NaCl, 1mM $CaCl_2$, 2 mM $MgCl_2$, 0.5% BSA; $5x10^6$ cells/ml). 50.000 cells were pre-incubated with/without blocking mAb (20 µg/ml) for 10 min at RT in a 96-wells V-shaped bottom plate. Ligand-coated fluorescent beads (20 beads/cell) and different stimuli/inhibitors were added and the suspension was incubated for 30 min at 37°C. The cells were washed and resuspended in 100 µl TSA. Adhesion was determined by measuring the percentage of cells, which have bound fluorescent beads, by flowcytometry using the FACScan (Becton and Dickinson & Co., Oxnard, CA).

Ex. 9F: Heterotypic cell clustering assays

Clustering between DC and ICAM-3 expressing cells was assessed by flowcytometry. DC and ICAM-3 expressing cells ($2x10^6$ cells/ml) were labeled respectively with sulfofluorescein (Molecular Probes, Eugene, OR; 50 µg/ml) and hydroethidine (Molecular Probes, Eugene, OR; 40 µg/ml) for 1 hour at 37°C. After washing, DC and the ICAM-3 expressing cells were mixed ($50x10^3$ cells each) and incubated at 37°C. At different time points the cells were fixed with

Ex. 9I: Immunohistochemical analysis

Cryosections (8 μm) of tonsils and lymph nodes were fixated in 100% aceton (10 min), washed with PBS and incubated with the first antibody (10 μg/ml) for 60 min at RT. After washing, the final staining was performed with the ABC-AP Vectastain kit (Vector Laboratories, Burlingame, CA) according to the manufacturer's protocol. Nuclear staining was performed with hematein ().

Table 1: Expression level of DC-SIGN on hematopoietic cells as determined by flowcytometric analyses and RT-PCR.

Cell-type	DC-SIGN expression*	DC-SIGN mRNA‡
monocytes	-	-
DC day 7	+++	+
PBL	-	-
T cells	-	-
B cells	-	-
B-cells (tonsils)$	-	n.d.
Thymocytes	-	-
Granulocytes	-	-
CD34+ cells	-	n.d.
PBMC (activated#)	-	-
T cell lines†	-	-
monocytic cell lines††	-	-

* mean fluorescence: -=<20, +++ >400 (staining with AZN-D1)
‡ RT-PCR with the DC-SIGN specific primers XF29 and XR1265 on total RNA isolated from the different cells
$ isolated from tonsils
# activated with PHA (10 μg/ml) and IL-2 (10 U/ml) for 2 days
† T cell lines : HSB, PEER, CEM and Jurkat
†† monocytic cell lines: THP-1, MM6 and U937
n.d., not determined

CAGGAGATCT ACCAGGAGCT GACCCGGCTG AAGGCTGCAG TGGGTGAGCT TCCAGAGAAA 420
TCTAAGCTGC AGGAGATCTA CCAGGAGCTG ACCTGGCTGA AGGCTGCAGT GGGTGAGCTT 480
CCAGAGAAAT CTAAGATGCA GGAGATCTAC CAGGAGCTGA CTCGGCTGAA GGCTGCAGTG 540
GGTGAGCTTC CAGAGAAATC TAAGCAGCAG GAGATCTACC AGGAGCTGAC CCGGCTGAAG 600
GCTGCAGTGG GTGAGCTTCC AGAGAAATCT AAGCAGCAGG AGATCTACCA GGAGCTGACC 660
CGGCTGAAGG CTGCAGTGGG TGAGCTTCCA GAGAAATCTA AGCAGCAGGA GATCTACCAG 720
GAGCTGACCC AGCTGAAGGC TGCAGTGGAA CGCCTGTGCC ACCCCTGTCC CTGGGAATGG 780
ACATTCTTCC AAGGAAACTG TTACTTCATG TCTAACTCCC AGCGGAACTG GCACGACTCC 840
ATCACCGCCT GCAAAGAAGT GGGGGCCCAG CTCGTCGTAA TCAAAAGTGC TGAGGAGCAG 900
AACTTCCTAC AGCTGCAGTC TTCCAGAAGT AACCGCTTCA CCTGGATGGG ACTTTCAGAT 960
CTAAATCAGG AAGGCACGTG GCAATGGGTG GACGGCTCAC CTCTGTTGCC CAGCTTCAAG 1020
CAGTATTGGA ACAGAGGAGA GCCCAACAAC GTTGGGGAGG AAGACTGCGC GGAATTTAGT 1080
GGCAATGGCT GGAACGACGA CAAATGTAAT CTTGCCAAAT TCTGGATCTG CAAAAAGTCC 1140
GCAGCCTCCT GCTCCAGGGA TGAAGAACAG TTTCTTTCTC CAGCCCCTGC CACCCCAAAC 1200
CCCCCTCCTG CGTAG 1215

2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 415 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Ser Asp Ser Lys Glu Pro Arg Leu Gln Gln Leu Gly Leu Leu
1 5 10 15

Glu Glu Glu Gln Leu Arg Gly Leu Gly Phe Arg Gln Thr Arg Gly
20 25 30

Tyr Lys Ser Leu Ala Gly Cys Leu Gly His Gly Pro Leu Val Leu
35 40 45

Gln Leu Leu Ser Phe Thr Leu Leu Ala Gly Leu Leu Val Gln Val
50 55 60

Asp Gly Ser Pro Leu Leu Pro Ser Phe Lys Gln Tyr Trp Asn Arg
335 340 345

Gly Glu Pro Asn Asn Val Gly Glu Glu Asp Cys Ala Glu Phe Ser
350 355 360

Gly Asn Gly Trp Asn Asp Asp Lys Cys Asn Leu Ala Lys Phe Trp
365 370 375

Ile Cys Lys Lys Ser Ala Ala Ser Cys Ser Arg Asp Glu Glu Gln
380 385 390

Phe Leu Ser Pro Ala Pro Ala Thr Pro Asn Pro Pro Pro Ala *
395 400 404

7. Use of a combination of: 1) a compound that binds to a C-type lectin on the surface of a dendritic cell; and attached thereto: 2) an antigen or a fragment or part thereof; in the preparation of a composition for modulating, in particular generating, increasing and/or promoting, an immune response in a animal, in particular a human or another mammal, against said antigen.

8. Use according to claim 7, in which the antigen is covalently bonded to or fused with the compound that can bind to the C-type lectin.

9. Use according to claim 7 or 8, in which the antigen is chosen from cancer antigens which can be used to generate an immune response against tumor cells that contain or express said antigen, or antigens (as used in vaccines against) infectious diseases,

10. Use according to any of claims 1-9, in which the compound that can bind to a C-type lectin on the surface of a dendritic cell is chosen from the group consisting of mannose carbohydrates such as mannan and D-mannose; fucose carbohydrates such as L-fucose; plant lectins such as concanavalin A; antibiotics such as pradimicin A; sugars such as N-acetyl-D-glucosamine and galactose; proteins such as gp120 and analogs or fragments thereof; and antibodies directed against a C-type lectin as expressed on the surface of a dendritic cell or a part, fragment or epitope thereof.

11. Use according to any of claims 1-9, in which the C-type lectin on the surface of a dendritic cell is a protein with the amino acid sequence of SEQ ID no.1, or a natural variant or equivalent thereof.

12. Use according to claim 10 or 11, in which the compound that can bind to a C-type lectin on the surface of a dendritic cell is a monoclonal antibody, preferably a monoclonal antibody directed against a C-type lectin with the amino acid sequence of SEQ ID no.1 or a natural variant or equivalent thereof; and/or a

thereof; and antibodies directed against a C-type lectin as expressed on the surface of a dendritic cell or a part, fragment or epitope thereof.

20. Use of an antibody according to claim 13 or 14 in the detection of dendritic cells in a biological sample.

21. Use of an antibody according to claim 13 or 14 in the isolation, preparation and/or purification of dendritic cells from a biological sample or a culture medium.

22. Use of an antibody according to claim 13 or 14 in an assay for determining the presence and/or the expression of C-type lectins, in particular a C-type lectin with the amino acid sequence of SEQ ID no.1 or a natural variant or equivalent thereof; and/or a part, fragment or epitope thereof, in a biological sample.

23. Method for producing, isolating and/or purifying dendritic cells from a biological sample or a culture medium, comprising the steps of:

a) contacting a biological sample or a culture medium that contains dendritic cells with an antibody according to claim 13 or 14;

b) separating the cells that bind to said antibody from cells that do not bind to said antibody, and optionally from any further constituents of the sample or medium;

and optionally further comprises the step of:

c) separating the cells that bind to the antibody from said antibody.

24. Method according to claim 23, in which the antibody is attached to a column or matrix, to (para)magnetic beads or to a similar solid support.

Fig 1a

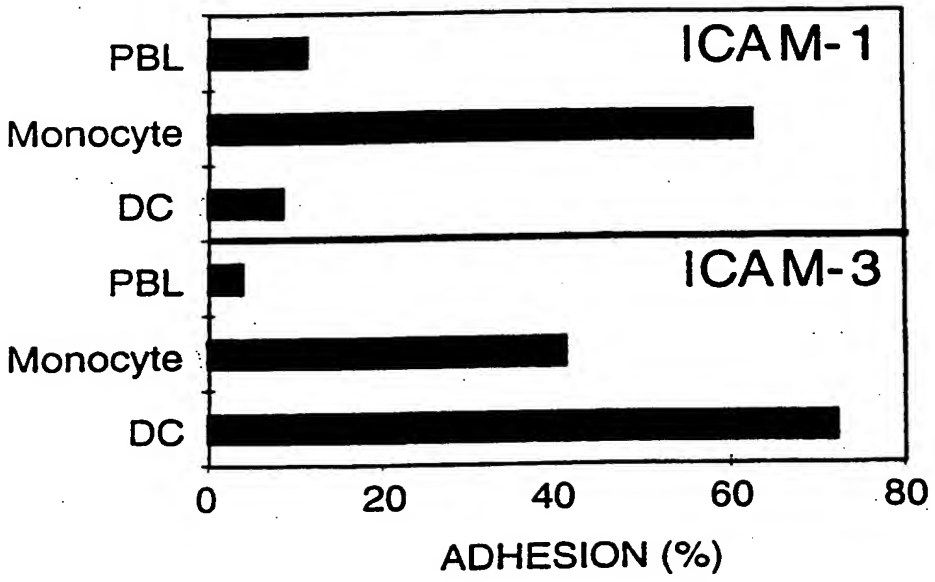


Fig 1b

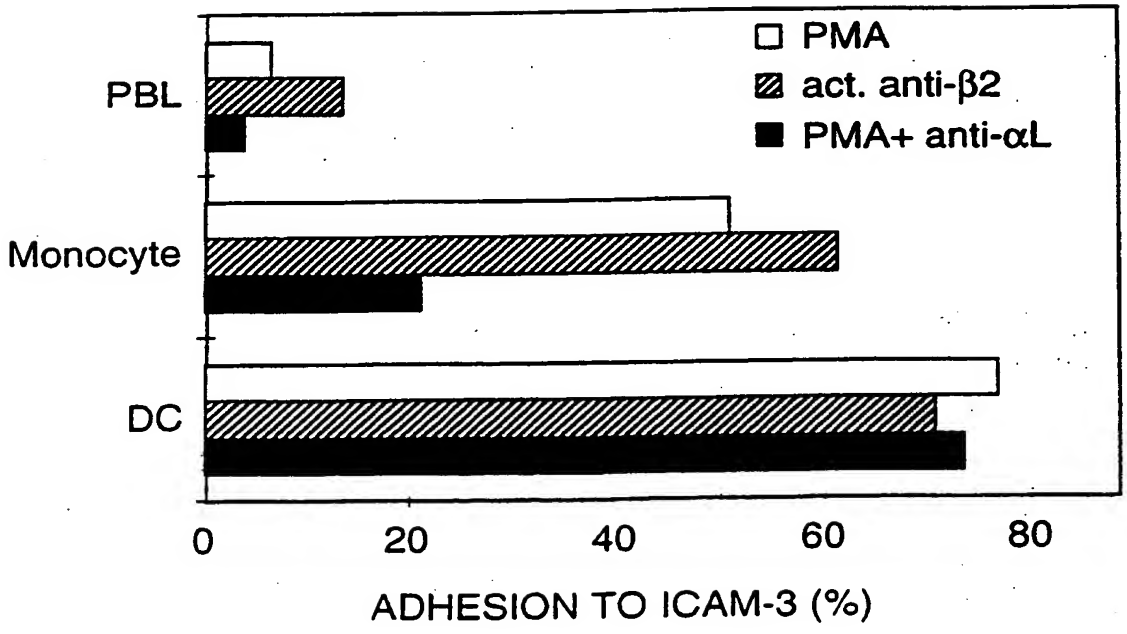
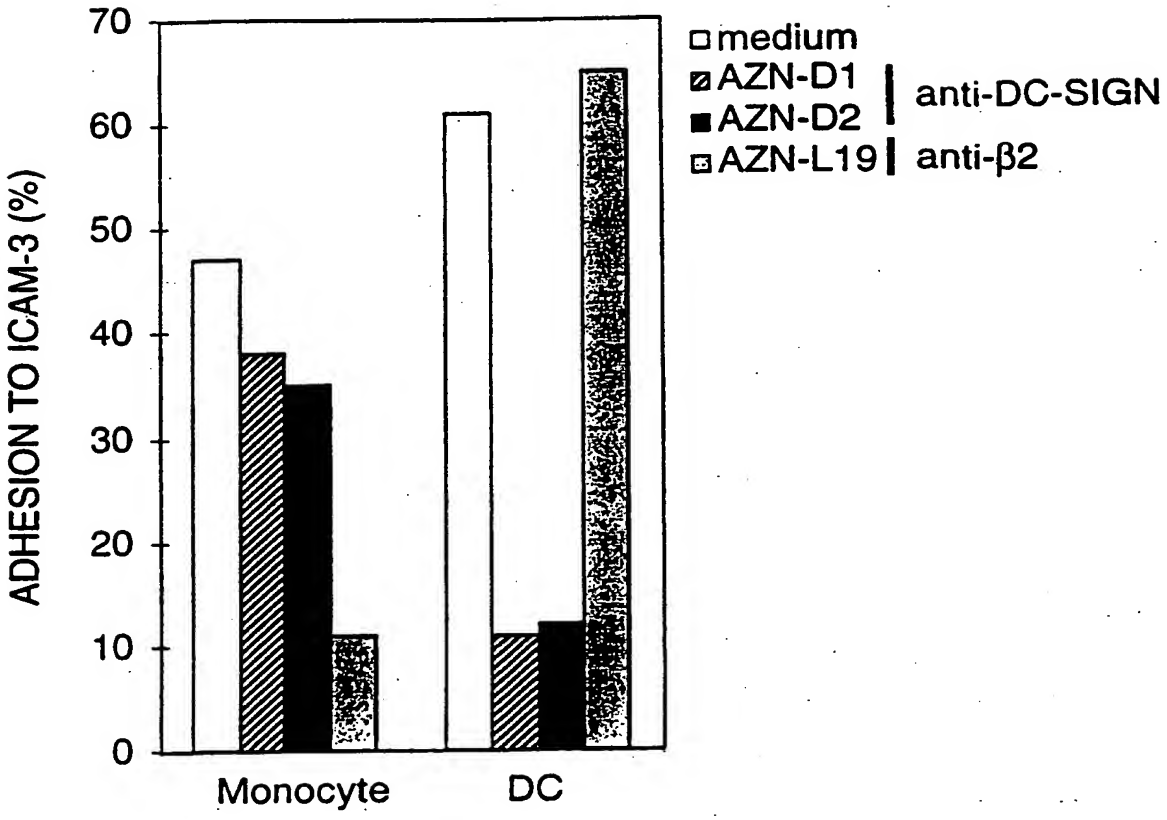


Fig 2a


70
60
50
40
30
20
10
0
ADHESION TO ICAM-3 (%)
Monocyte
DC
medium
AZN-D1
AZN-D2
AZN-L19
anti-DC-SIGN
anti-β2

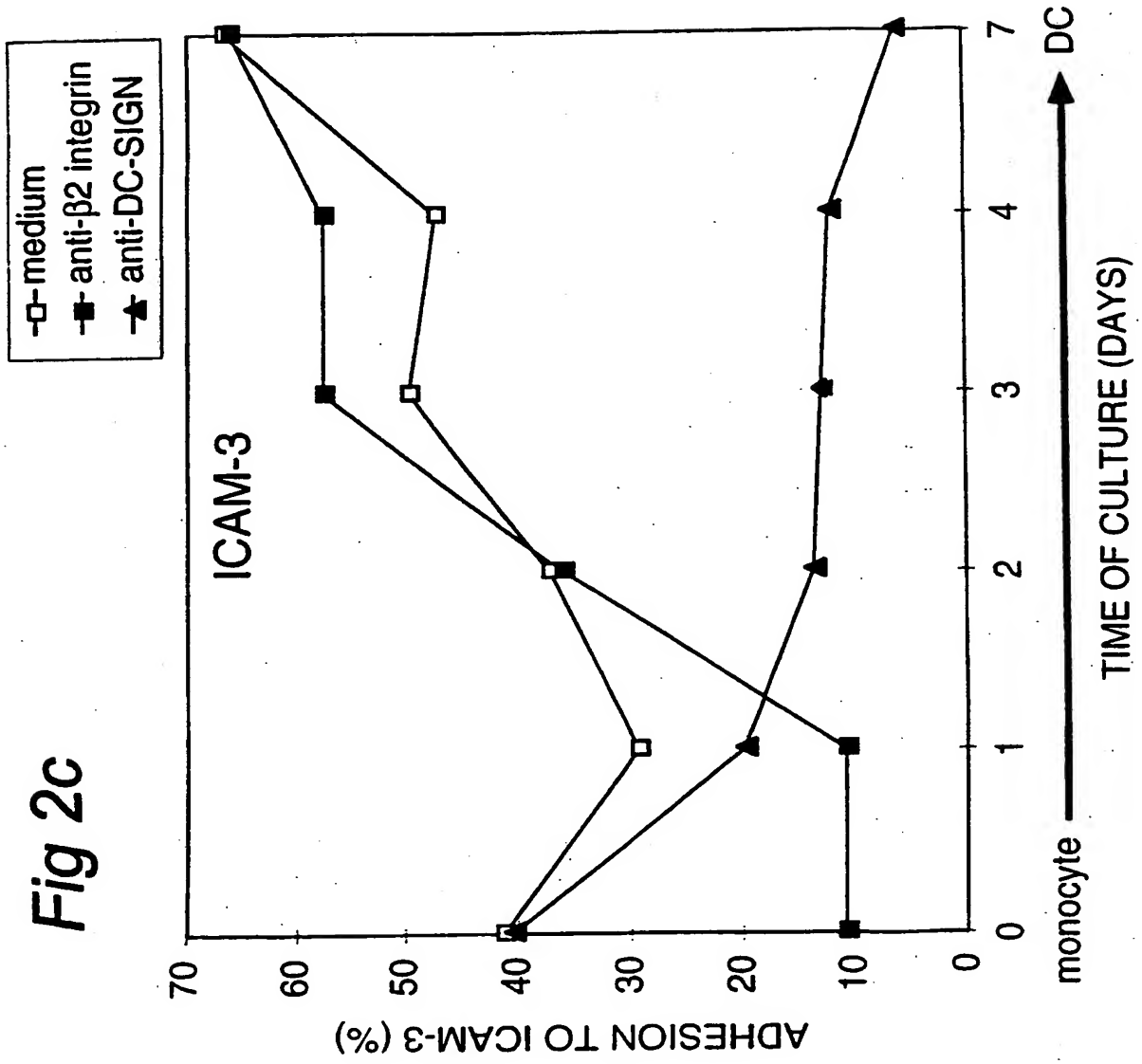

medium
anti-β2 integrin
anti-DC-SIGN
ICAM-3
ADHESION TO ICAM-3 (%)
0
10
20
30
40
50
60
70
0
1
2
3
4
7
monocyte
DC
TIME OF CULTURE (DAYS)

Fig 2c

Fig 3a

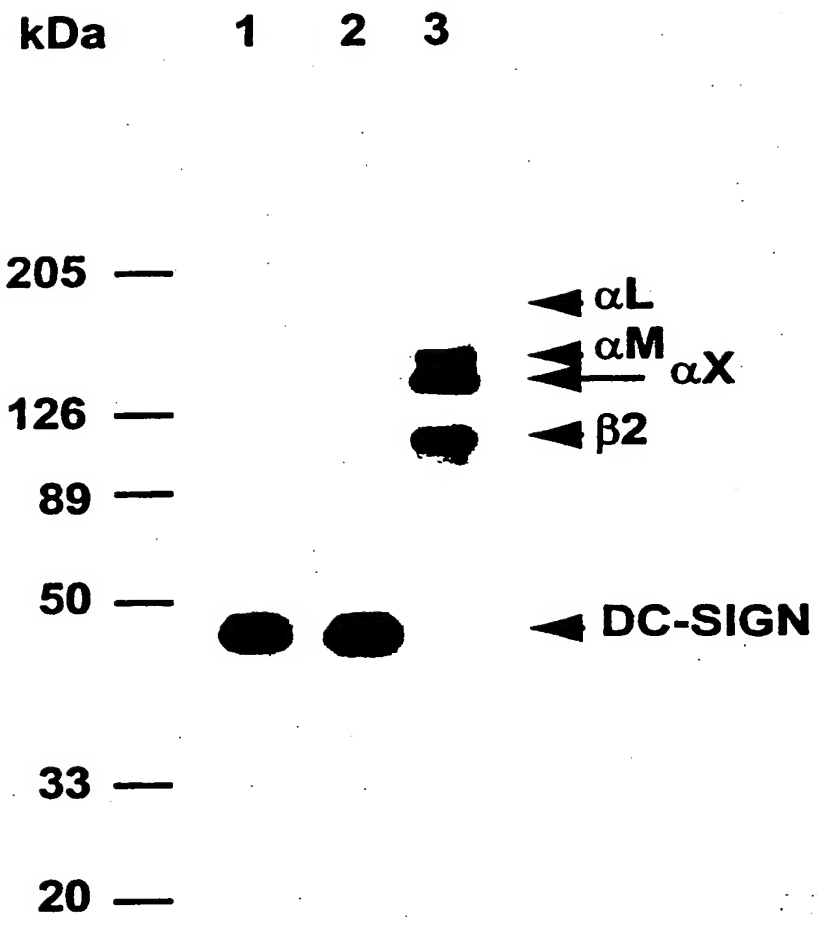


Fig 5

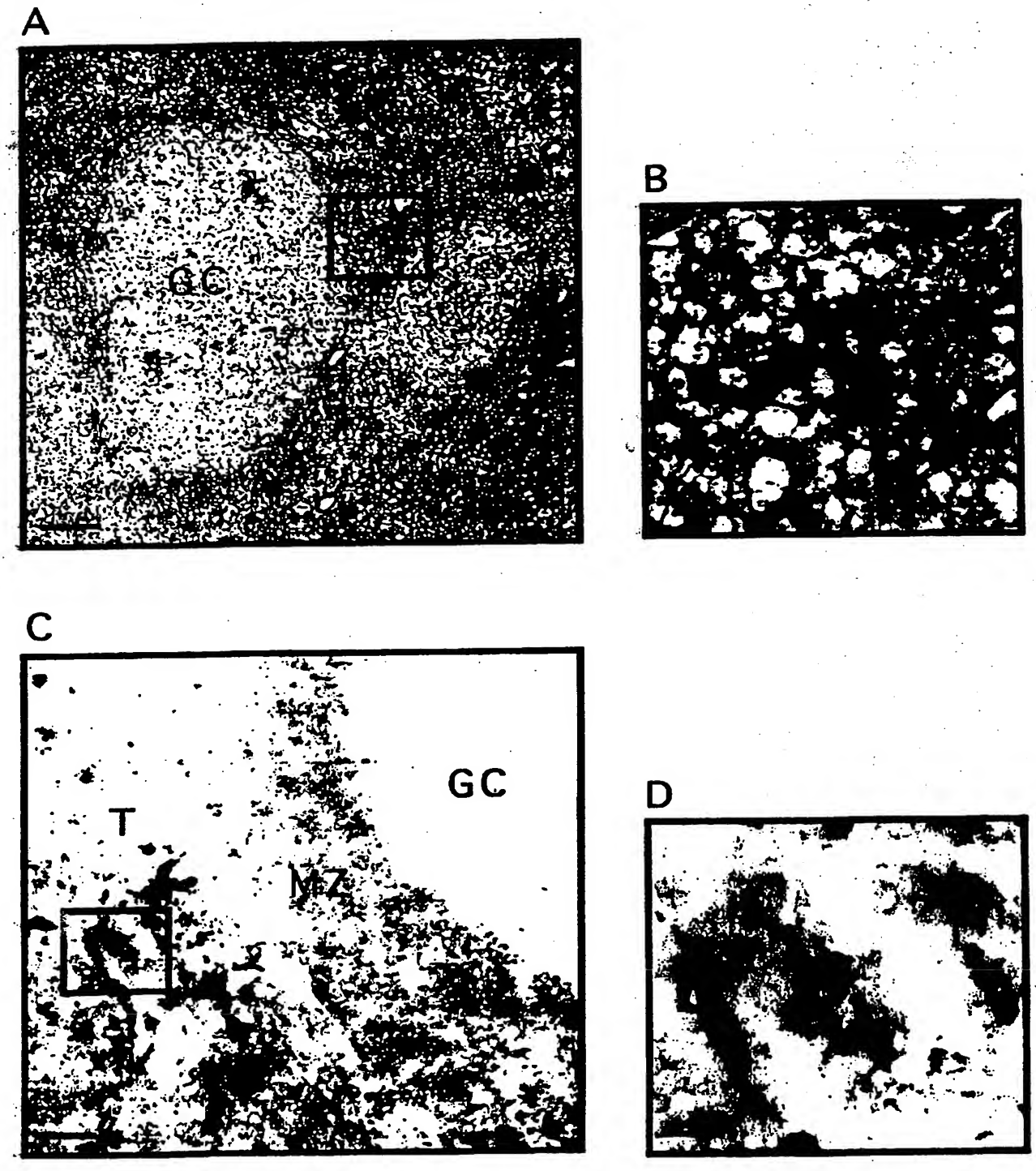


Fig 6b

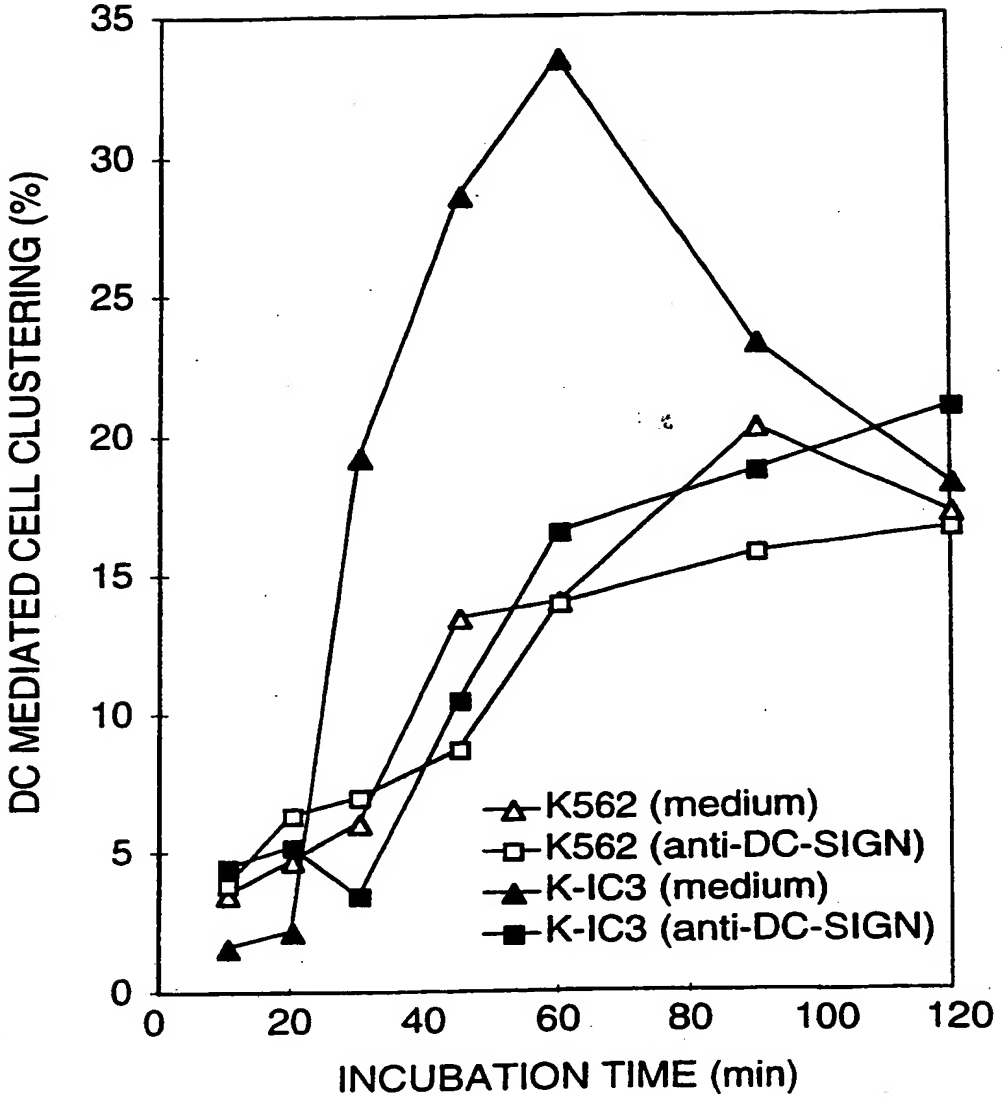
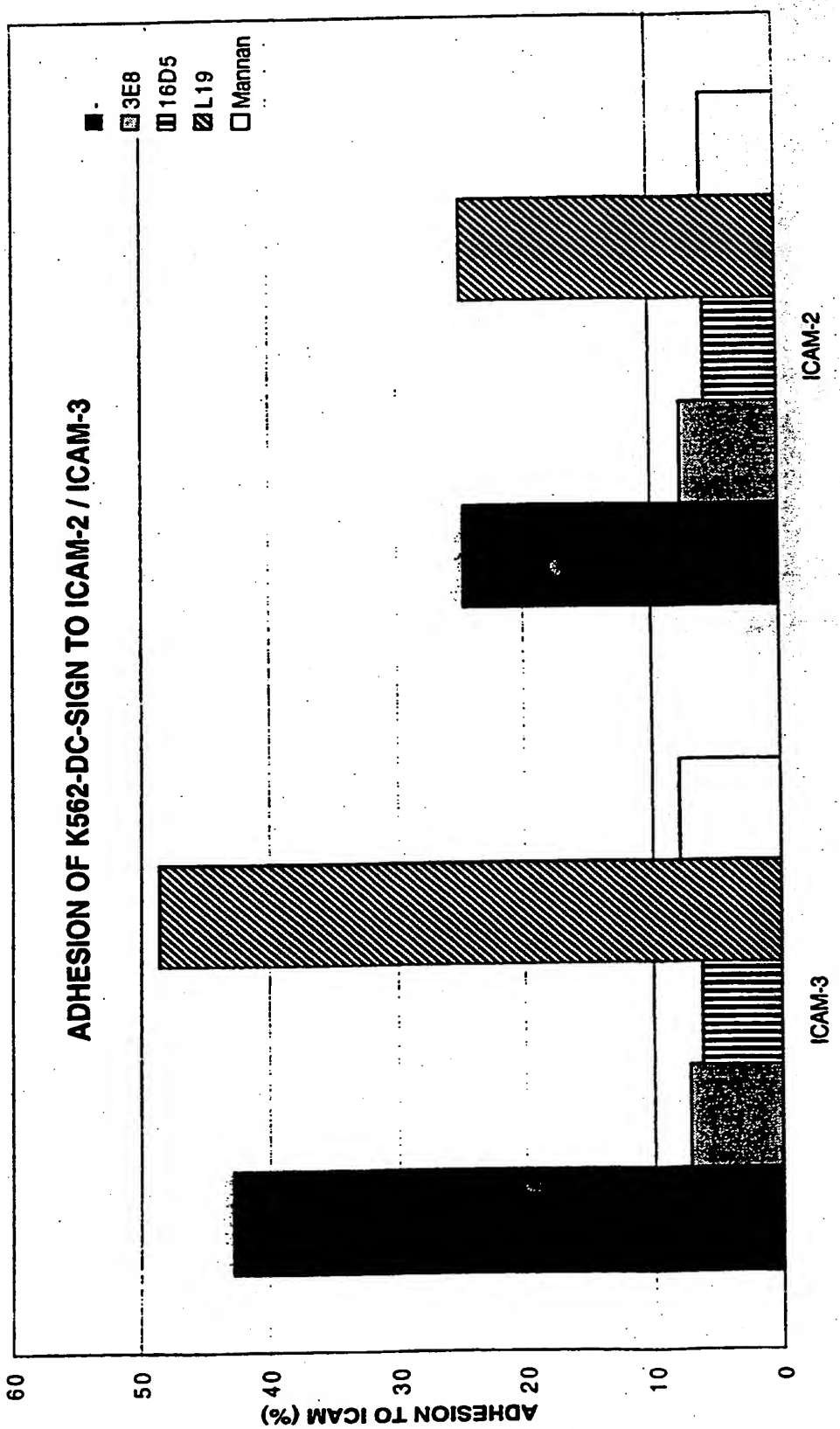


Fig 8


ADHESION OF K562-DC-SIGN TO ICAM-2 / ICAM-3
-
3E8
16D5
L19
Mannan
ICAM-2
ICAM-3
ADHESION TO ICAM (%)
0
10
20
30
40
50
60

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: Koninklijke Universiteit Nijmegen, Afdeling tumorimmunologie
(B) STREET: Philips van Leydenlaan 25
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(F) POSTAL CODE (ZIP): 6525 EX
(G) TELEPHONE: + 31 243 617 600
(H) TELEFAX: + 31 243 540 339

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(ii) TITLE OF INVENTION: COMPOSITION AND METHOD FOR MODULATING DENDRITIC CELL-T CELL INTERACTION

(iii) NUMBER OF SEQUENCES: 2

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1215 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ATGAGTGACT CCAAGGAACC AAGACTGCAG CAGCTGGGCC TCCTGGAGGA GGAACAGCTG 60

AGAGGCCTTG GATTCCGACA GACTCGAGGA TACAAGAGCT TAGCAGGGTG TCTTGGCCAT 120

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Ser Asp Ser Lys Glu Pro Arg Leu Gln Gln Leu Gly Leu Leu
1 5 10 15

Glu Glu Glu Gln Leu Arg Gly Leu Gly Phe Arg Gln Thr Arg Gly
20 25 30

Tyr Lys Ser Leu Ala Gly Cys Leu Gly His Gly Pro Leu Val Leu
35 40 45

Gln Leu Leu Ser Phe Thr Leu Leu Ala Gly Leu Leu Val Gln Val
50 55 60

Ser Lys Val Pro Ser Ser Ile Ser Gln Glu Gln Ser Arg Gln Asp
65 70 75

Ala Ile Tyr Gln Asn Leu Thr Gln Leu Lys Ala Ala Val Gly Glu
80 85 90

Leu Ser Glu Lys Ser Lys Leu Gln Glu Ile Tyr Gln Glu Leu Thr
95 100 105

Gln Leu Lys Ala Ala Val Gly Glu Leu Pro Glu Lys Ser Lys Leu
110 115 120

Gln Glu Ile Tyr Gln Glu Leu Thr Arg Leu Lys Ala Ala Val Gly
125 130 135

Glu Leu Pro Glu Lys Ser Lys Leu Gln Glu Ile Tyr Gln Glu Leu
140 145 150

Thr Trp Leu Lys Ala Ala Val Gly Glu Leu Pro Glu Lys Ser Lys
155 160 165

Met Gln Glu Ile Tyr Gln Glu Leu Thr Arg Leu Lys Ala Ala Val
170 175 180

Gly Glu Leu Pro Glu Lys Ser Lys Gln Gln Glu Ile Tyr Gln Glu
185 190 195

Leu Thr Arg Leu Lys Ala Ala Val Gly Glu Leu Pro Glu Lys Ser
200 205 210

Lys Gln Gln Glu Ile Tyr Gln Glu Leu Thr Arg Leu Lys Ala Ala
215 220 225

Val Gly Glu Leu Pro Glu Lys Ser Lys Gln Gln Glu Ile Tyr Gln
230 235 240

Glu Leu Thr Gln Leu Lys Ala Ala Val Glu Arg Leu Cys His Pro
245 250 255

Cys Pro Trp Glu Trp Thr Phe Phe Gln Gly Asn Cys Tyr Phe Met
260 265 270

INTERNATIONAL SEARCH REPORT

Intern al Application No
PCT/NL 00/00253

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K16/28 A61K39/395 C12N5/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
STRAND, EPO-Internal, WPI Data, PAJ, BIOSIS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 93 01820 A (SQUIBB BRISTOL MYERS CO) 4 February 1993 (1993-02-04) cited in the application see Figure 3A. the whole document ---	11-13,22
X	CURTIS B.M. ET AL.,: "Sequence and expression of a membrane-associated C-type lectin that exhibits CD4-independent binding of human immunodeficiency virus envelope glycoprotein gp 120" PROC. NATL. ACAD. SCI. U.S.A., vol. 89, 1992, page 8356-8360 XP002118177 cited in the application figure 2 --- -/--	11-13,22

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 July 2000	02/08/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Mateo Rosell, A.M.

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Intern al Application No
PCT/NL 00/00253

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	TSUNETSUGU-YOKOTA Y. ET AL.: "Efficient virus transmission from dendritic cells to CD4+ T cells in response to antigen depends on close contact through adhesion molecules" VIROLOGY, vol. 239, 1997, pages 259-268, XP002118178 page 259, paragraph 1 -page 260, right-hand column, paragraph 1 see discussion ---	1,2,4-6
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